IRIS AperTO

UNIVERSITÀ DEGLI STUDI DI TORINO

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Early effects of first-line treatment with anti-interleukin-6 receptor antibody tocilizumab for chronic active antibody-mediated rejection in kidney transplantation

This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/1741500 since 2020-06-16T14:20:27Z

Published version:

DOI:10.1111/ctr.13908

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

14 December 2021

Check for updates

Accepted Article

PROF. LUIGI BIANCONE (Orcid ID : 0000-0002-1900-1156)

Article type : Original Article

Early effects of first-line treatment with anti-interleukin-6 receptor antibody tocilizumab for chronic active antibody-mediated rejection in kidney transplantation

Antonio Lavacca[1] MD, Roberto Presta[1] MD, Chiara Gai[2] PhD, Alberto Mella[1] MD, PhD, Ester Gallo[1] MD, Giovanni Camussi[2] MD, Isabella Abbasciano[1] MD, Antonella Barreca[3] MD, PhD, Cristiana Caorsi[4] MD, Fabrizio Fop[1], Maria Messina[1] MD, Maura Rossetti[1] MD, and Luigi Biancone[1] MD, PhD

[1] Renal Transplant Center "A. Vercellone", Nephrology, Dialysis and Renal Transplant Division, "Città della Salute e della Scienza" Hospital, Department of Medical Sciences, University of Turin, Turin, Italy

[2] Stem Cell Laboratory, Department of Medical Sciences, University of Turin, Turin, Italy

[3] Division of Pathology, "Città della Salute e della Scienza" Hospital, Department of Medical Sciences, University of Turin, Turin, Italy

[4] Immunogenetic and Transplant Biology Center, "Città della Salute e della Scienza" Hospital and Department of Medical Sciences, University of Turin, Italy

Running head: tocilizumab for cAMR in renal transplantation

Corresponding Author: Prof. Luigi Biancone, MD, PhD

Phone number +390116335594

Fax number +390116963158

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/CTR.13908

This article is protected by copyright. All rights reserved

E-mail luigi.biancone@unito.it; luigi.biancone@gmail.com
Postal address: Renal Transplantation Unit “A. Vercellone”, Division of Nephrology Dialysis and Transplantation, Città della Salute e della Scienza Hospital and Department of Medical Sciences, University of Turin, Corso Bramante 88, 10126 Turin, Italy
ORCID ID 0000-0002-7700-6350

Accepted Article

This article is protected by copyright. All rights reserved

Lavacca A, Presta R, Gai C, Mella A, Gallo E, Camussi G, Abbasciano I, Barreca A, Caorsi C, Fop F, Messina M, Rossetti M, and Biancone L

Clin Transplant

ABSTRACT

Introduction Chronic active antibody-mediated rejection (cAMR) is a major determinant of late allograft failure. Rituximab/immunoglobulins (IVIg) + plasma exchange (PLEX) showed controversial results in cAMR treatment. Tocilizumab (TCZ), a humanized anti-interleukin 6 receptor antibody, has been recently used as rescue therapy in patients non-responsive to rituximab/IVIg/PLEX with favorable outcomes. Whether TCZ acts "per se" or requires a priming effect from previous treatments is currently unknown.

Methods 15 patients with cAMR were treated with TCZ as a first-line therapy and followed for a median time of 20.7 months.

Results Despite the majority of patients experiencing advanced transplant glomerulopathy (TG) at diagnosis (60% with cg3), glomerular filtration rate and proteinuria stabilized during the follow-up, with a significant reduction in donor-specific antibodies. Protocol biopsies after 6 months demonstrated significant amelioration of microvascular inflammation and no TG, C4d deposition or IF/TA progression. Gene-expression and immunofluorescence analysis showed upregulation of three genes (TJP-1, AKR1C3 and CASK) involved in podocyte, mesangial and tubular restoration.

Conclusion TCZ adopted as a first-line approach in cAMR was associated with early serological and histological improvements and functional stabilization even in advanced TG, suggesting a role for the use of TCZ alone with the avoidance of unnecessary previous immunosuppressants.

KEY WORDS: Tocilizumab; Chronic antibody-mediated rejection; Transplant glomerulopathy; Kidney transplantation; donor specific antibodies; anti-HLA antibody; angiotensin II type I-receptor antibody

CORRESPONDING AUTHOR: Prof. Luigi Biancone. Renal Transplantation Unit "A. Vercellone", Division of Nephrology Dialysis and Transplantation, Città della Salute e della

This article is protected by copyright. All rights reserved

Scienza Hospital and Department of Medical Sciences, University of Turin, Corso Bramante 88, 10126 Turin, Italy

ABBREVIATIONS

AEs/SAEs	adverse events/severe adverse events
AKR1C3	aldo-keto reductase family 1 member C3
AT_1R-Abs	anti–angiotensin type 1 receptor antibodies
cAMR	chronic active antibody-mediated rejection
CASK	calcium/calmodulin dependent serine protein kinase
CNI	calcineurin inhibitors
CTR	healthy controls
DSAs/iDSAs	donor-specific antibodies/immunodominant donor-specific antibodies
eGFR	estimated glomerular filtration rate
ENDATs	endothelial transcripts
IF/TA	interstitial fibrosis/tubular atrophy
IL-6	interleukin 6
IQR	interquartile range
IVIg	intravenous immunoglobulins
MMF/MPA	Mycophenolate Mofetil/Mycophenolic Acid
RQ	relative quantification
PLEX	plasma exchange
TCZ	tocilizumab
TG	transplant glomerulopathy
TJP-1	tight junction protein-1

This article is protected by copyright. All rights reserved

Accepted Article

INTRODUCTION

Long-term graft survival remains a major challenge in kidney transplantation. A leading cause of late allograft failure is chronic active antibody mediated rejection (cAMR), mostly due to anti-HLA donor-specific antibodies (DSA)[1]. Different approaches have been adopted to reduce the production and the detrimental effect of DSAs. Initial studies showed a benefit from the use of the anti-CD20 monoclonal antibody rituximab in combination with high-dose intravenous immunoglobulins (IVIg)[2–4], but recent experiences, including our case series, have not confirmed these results, even with the addition of plasmapheresis (PLEX)[5–7]. More recently, a multicenter randomized trial comparing PLEX/IVIg with or without rituximab did not find any benefit of rituximab on cAMR outcomes[8]. Other therapies such as the anti-proteasome inhibitor bortezomib[9] or anti-C5b-9 eculizumab[10] were studied in randomized trials without effect.

In 2017 Choi et al reported favorable effects in 36 patients with cAMR unresponsive to rituximab/PLEX/IVIg after 2 years of treatment with tocilizumab (TCZ), a humanized monoclonal antibody against the interleukin 6 (IL-6) receptor[11]. In this study DSA levels declined with renal function stabilization, improvement in microvascular inflammation (g + ptc and C4d scores) and no progression of transplant glomerulopathy (cg score) at a 1-year protocol biopsy.

It is currently unknown whether the effects of TCZ as a rescue therapy after rituximab were primed by B-cell depletion before cytokine interference. Indeed, the cumulative effect of multiple lines of therapy may potentially impact safety, particularly considering chronic treatments such as TCZ[12,13]. In addition, the continuous change in the demography of recipients toward an older age and a frail profile[14–16] may considerably influence the risk/benefit balance of increasing long-term immunosuppressive load. In this context avoidance of unnecessary treatments is mandatory.

In the present study we report our experience in 15 cAMR patients with severe transplant glomerulopathy (TG) treated with TCZ as first-line therapy with analysis of functional, serological and histological results.

This article is protected by copyright. All rights reserved

MATERIALS AND METHODS

Patients and study design

We performed a single-center, open-label, non-sponsored case study at the Division of Nephrology, Dialysis and Transplantation of Città della Salute e della Scienza di Torino, University Hospital. The study was approved by both the internal Ethical and Pharmaceutical Committees. TCZ treatment was funded by the Italian Medicines Agency - Ministry of Health (AIFA) through the AIFA 5% fund.

Between 2016 and 2018, 15 patients with cAMR according to Banff criteria[17] and without any previous therapy for cAMR, including rituximab, IVIg, PLEX, high dose steroids, bortezomib, anti-thymoglobulin or complement blockers, were treated with TCZ. One patient was treated with pulse steroids/IVIg/rituximab/PLEX as part of a desensitization protocol for an ABO-incompatible donor 6 years prior to the cAMR diagnosis and subsequent TCZ treatment, and one patient (6.7%) had a previous acute AMR episode treated with pulse steroids and PLEX/IVIg 30 months before cAMR. All biopsies were reviewed by a single pathologist with 20-years of experience in kidney allograft histology. All patients gave written informed consent after description of the potential risks and benefits of TCZ. Before the initiation of TCZ, patients were tested for viral infections (HBV, HCV, HIV) and for exposure to tuberculosis using the purified protein derivative skin test or QuantiFERON-TB test. Negative chest x-ray, abdomen ultrasound scan and cardiological assessment were required before starting treatment.

TCZ was given at a dose of 8 mg/kg (maximum of 800 mg) after premedication with steroids, paracetamol and chlorphenamine in our Ambulatory Transplantation Department with constant monitoring during the 60 min infusion time. Doses were scheduled monthly, and TCZ was continuously maintained if no adverse events (AEs) were recorded.

After initiation of therapy, patients were closely monitored for renal function, proteinuria, graft and patient survival, DSA levels (anti-HLA and anti–angiotensin type 1 receptor antibodies [AT_1R-Ab]) and AEs and severe AEs. In order to evaluate early histological changes, a protocol graft biopsy was performed approximately 6 months after TCZ initiation (median 7.7 months; 6.8-8.6).

For immunosuppressive maintenance, all patients were treated with a calcineurin inhibitor (CNI)-based immunosuppressor (20.0% cyclosporine A, 80.0% tacrolimus) plus mycophenolate mofetil/mycophenolic acid (MMF/MPA, 46.7%); 10 patients (66.7%) were also on steroids at the baseline. After diagnosis of cAMR, therapy was cautiously modified either by introducing

This article is protected by copyright. All rights reserved

MMF/MPA and/or steroids (with contemporary suspension of mTOR inhibitor, if used) or switching from cyclosporine A to tacrolimus (TAC) in patients who were not already on TAC/MMF/steroids (12/15, 80.0%). In one patient with severe graft dysfunction, conversion to belatacept with maintenance of low-dose tacrolimus (target range 2-3 ng/mL) was performed 7.9 months before TCZ initiation.

Serology

Sera were evaluated both at the time of the index biopsy and the 6-month protocol biopsy. All samples were tested using a Luminex platform and commercially available SAB kits (LABScreen, One Lambda Inc, Canoga Park, CA, USA) in order to identify HLA Class I and II IgG DSA. The cut-off was set at the normalized mean fluorescence intensity (MFI) value of 1000. We also tested AT_1R-Ab IgG using a commercial enzyme-linked immunosorbent assay (ELISA) kit (One Lambda Inc, Canoga Park, CA, USA).

Gene expression analysis

In gene expression analysis we evaluated the baseline and 6-month protocol biopsy in 11 transplanted patients with normal histology and similar clinical characteristics (control group). For each sample, total RNA was extracted from 3 pieces of 10 μm sections of Serra's fluid-fixed, paraffin-embedded samples by PureLink FFPE Total RNA Isolation Kit (Invitrogen, Thermo Fisher Scientific) according to the manufacturer's instructions. RNA concentration was assessed using a MySpec instrument (VWR), and cDNA was produced from 400 ng of total RNA using the Super Script IV VILO Master Mix with ezDNase enzyme (Invitrogen, Thermo Fisher Scientific). In addition, the expression profile of a panel of 43 endothelial, mesangial and podocyte genes directly involved in antibody mediated rejection and/or glomerulonephritis [Supplementary Table 1; references[10,18–23]] was also tested. Gene expression for each target gene and five endogenous controls was measured using a specific TaqMan® Gene Expression Assay (Thermo Fisher Scientific) spotted on a custom TaqMan® Array microfluidic card (Thermo Fisher Scientific). The samples were mixed with TaqMan® Fast Advanced Master Mix (Thermo Fisher Scientific), and TaqMan® Array microfluidic cards were prepared according to the manufacturer's instructions and run using the QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific). Each sample was run in duplicate. Retrotranscription negative controls (no retrotranscription enzyme) were run for 4 samples. A no-template control (no cDNA) was run once on a TaqMan® Array

This article is protected by copyright. All rights reserved

microfluidic card. Both negative controls showed no amplification at Ct < 37. ExpressionSuite Software 1.0.3 (Thermo Fisher Scientific) was used to calculate relative quantification (RQ) values by the 2-ΔΔCt method. Values of Ct > 37 or Cq conf < 0.6 were excluded from analysis. 18s and NPHS2 were set as endogenous controls for multiple endogenous control analysis. Data were normalized to the expression level of healthy controls (CTR).

Immunofluorescence assay

Serial separated sections of frozen tissue from pre- and post-treatment biopsies (available in 6 patients) were fixed in cold acetone for 10 min, washed with 0.1% PBS-BSA, blocked with 1% PBS-BSA and subsequently incubated with mouse monoclonal IgG1 anti-human tight junction protein 1 (TJP1) FITC-conjugated (Thermo Fisher Scientific, Waltham, MA, USA) (5 µg/ml), rabbit polyclonal anti-aldo-keto reductase family 1 member C3 (AKR1C3) (Thermo Fisher Scientific, Waltham, MA, USA) (1:250), rabbit polyclonal anti-calcium/calmodulin dependent serine protein kinase (CASK) (Thermo Fisher Scientific, Waltham, MA, USA) (1:20) and a secondary fluorescein anti-rabbit antibody (goat anti-rabbit IgG Alexa Fluor® 594, Thermo Fisher Scientific, Waltham, MA, USA) (1:500) for 1h at room temperature. The sections were washed again, nuclear stained with Hoechst dye 33258 2.5 µg/mL (Sigma-Aldrich, St. Louis, MO, USA) mounted and observed under a confocal microscope (Leica SP5, Leica Microsystems, Mannheim, Germany). Digital images were processed using Fiji-ImageJ software[23].

Statistical analysis

Statistical analysis was performed with SPSS (IBM SPSS Statistics, vers. 25.0.0). Continuous variables are presented as mean ± standard deviation or as median and interquartile range (IQR), according to their distribution. Normal distribution was analyzed using a Kolmogorov-Smirnov test. The differences between before and after observations were analyzed using a paired Student's t-test or Wilcoxon test. Categorical variables are presented as fractions. Gene expression analysis was performed using GraphPad Prism 6.01 software on RQ values using a nonparametric Kruskal-Wallis test. Significance level was set at $\alpha < 0.05$ for all tests.

This article is protected by copyright. All rights reserved

RESULTS

Baseline characteristics

Table 1 shows the baseline characteristics of our population. Twelve out of 15 patients (80.0%) received a deceased-donor kidney transplant, and 3/15 (20.0%) received a kidney from an extended-criteria donor according to Crystal City criteria[28]. Two out of 15 (13.3%) were retransplants. Most patients were men (12/15, 80.0%) with a median age at the time of transplantation of 38.3 years (23.0-47.5). The median number of HLA mismatches was 4 (3-4.5). The median vPRA was 3.5% (0-83.75), with 3 patients displaying a total vPRA>80%.

All patients had HLA-DSAs, mostly anti-class II and de novo (14/15, 93.3%). In patients with available sera, 11/13 (84.6%) also showed elevated levels of AT_1R-Ab at the time of cAMR diagnosis.

cAMR was diagnosed by a for-cause biopsy at a median time of 5.9 years (4.6-14.0) after transplantation, and TCZ was started at a median time of 100 days (79-137.5) after the biopsy. At diagnosis most of the patients experienced both severe transplant glomerulopathy (median cg score 3, 2-3) and microvascular inflammation (g + ptc score 3, 2-4).

Functional, histological and serological modifications after TCZ

Patients were monitored for a median time of 20.7 months (18.0-27.8, max 38.1 months) from the first TCZ dose. Graft loss was observed in only one patient (6.7%), 30 months after diagnosis and 25.3 months after initiation of TCZ treatment. Considering the entire population, both estimated glomerular filtration rate (eGFR) and 24-hour proteinuria showed stabilization at the 12 month follow up (Fig. 1A-B). eGFR declined by 10.5 ml/min/1.73m^2 (median) in the 12 months before cAMR diagnosis compared to 4.4 ml/min/1.73m^2 the first year after diagnosis. Median proteinuria at diagnosis and at the 12 month follow up were 1.1 and 1 g/day, respectively.

These results were combined with a significant reduction in microvascular inflammation (g + ptc score 3, 2-4 pre-TCZ vs 2; 1-2.5 post-TCZ; p=0.014) and an absence of progression in chronicity scores (cg and IF/TA) or C4d deposition (Table 2, Fig. 2A-B). In two cases with relevant interstitial edema at the index biopsy we also observed a remarkable decrease in inflammation after TCZ treatment.

Figure 3A-B and Table 2 display the impact of TCZ on immunodominant anti-HLA-DSA (iDSA) levels in this population. iDSAs were defined as DSAs with highest MFI detected in patients' sera. Mean MFI values significantly declined after TCZ treatment (22600, 21700-23700

This article is protected by copyright. All rights reserved

pre-TCZ and 18200, 12650-22150 post-TCZ; p = 0.002), with complete negativization in one patient. This trend was also confirmed for AT_1R-Ab (Fig. 3C). After 6 months treatment with TCZ, median AT_1R-Ab levels were significantly reduced (15.8, 12.5-16.6 U/mL pre-TCZ and 8.4, 6.8-11.3 post-TCZ; p = 0.003) with negativization in six patients.

These results appear to be further confirmed in an additional cohort of 13 patients with TG currently in treatment with TCZ in our center, in which there was no graft failure in DSA positive (anti-HLA/AT_1R-Ab) subjects (data not shown).

Gene expression analysis and immunofluorescence assay pre- and post-TCZ

We compared baseline gene expression values at cAMR diagnosis to the control group and post-TCZ protocol biopsies. We observed an upregulation of ENDATs in cAMR patients compared to the control group but no differences before and after treatment with TCZ, a finding that may reflect the short period of observation (Fig. 4A). On the other hand, we identified three other genes differentially expressed before and after treatment with TCZ in the panel of mesangial and podocyte-related genes (Figure 4B). TJP-1 and AKR1C3 showed a non-significant reduction at baseline compared to controls but were significantly upregulated after treatment with TCZ compared to baseline. CASK was significantly downregulated at baseline compared to the control group and was significantly upregulated after treatment.

These trends were confirmed by immunofluorescence assay, with an increase in mean intensity after treatment for TJP-1 (16.336 vs 27.367 AU) and AKR1C3 (5.785 vs 8.778 AU) (Fig. 5A-D). CASK mean intensity was similar before and after treatment (8.021 vs 7.713 AU), although some patients showed a moderate increase (Fig. 5E-F).

Adverse events

During the follow-up, 5 patients (33.3%) experienced bacterial infections (4 in the urinary tract, and 1 in the lower respiratory tract) which were promptly resolved with medical therapy, and no hospitalization or cessation of TCZ therapy were needed. In one patient an encephalitis of undefined origin was observed three months after TCZ initiation. Although the event was not related to TCZ and all serological, virological (including JC, Toscana and West Nile viruses) and bacteriological tests on blood and cerebrospinal fluid were negative, the drug was temporarily suspended and restarted after complete clinical recovery. Two years later, the same patient developed bacterial pneumonia, and TCZ was stopped until the infection was resolved. Two

This article is protected by copyright. All rights reserved

patients developed interstitial lung disease. In one patient adenovirus was detected on bronchoalveolar lavage, so TCZ was temporarily suspended and restarted after complete recovery. In the other patient microbiological tests were negative, and a causal relationship with TCZ was suspected, so the therapy was stopped completely. Four patients (26.7%) developed hypogammaglobulinemia (< 450 mg/dl) during TCZ therapy, and IVIg replacement was required in one of them. Three patients (20.0%) had asymptomatic mild alterations in liver enzymes, and one had an increase in pancreatic enzymes. In these cases, TCZ was maintained, and tests were repeated with no further deterioration.

Accepted Article

This article is protected by copyright. All rights reserved

DISCUSSION

There are few treatment options for cAMR in the field of renal transplantation. Graft loss is predominantly related to this condition[24,25], and improvement has been debatable after the adoption of commonly considered "standard-of-care" therapies (PLEX, IVIg, rituximab), but also with novel approaches (bortezomib, eculizumab)[9,10,26]. Adverse effects are not observed in desensitization protocols that adopt rituximab with IVIg[27]; however, rituximab treatment is also associated with higher risk of infectious complications that may be particularly insidious in immunosuppressed patients[26,28–30], further bringing into question its use in cAMR based on its risk-benefit ratio.

The anti-IL-6 receptor antibody TCZ has been used as a rescue therapy for cAMR after failure of rituximab/IVIg with or without PLEX with favorable results in reducing alloantibodies, ameliorating rejection lesions and slowing renal function deterioration[11,31]. Extension of this case series and some preliminary reports from other groups support these findings[32–35]. However, the individual role of IL-6 pathway blockade in treating cAMR with TCZ following failure of rituximab treatment has not been identified. Even if those treatments were not concomitant, it is well known that even a single dose of rituximab may exert long-term impact in kidney transplanted patients[36]. On these bases, one can speculate that the observed effect of TCZ may require as "priming" a first-line treatment with rituximab. The depletion of mature B-cells may theoretically synergize with the interfering effect of TCZ in B-cell maturation and T-B cell interaction. On the other hand, in vitro studies have shown that rituximab may induce IL-6 secretion by resting and activated B cells[37], a notion that may partially explain the lack of DSA level reduction after rituximab[8] but not after TCZ[31].

Our study shows that TCZ as a first-line treatment is associated with functional, serological and histological effects mirroring those observed by Choi et al[11]. Additionally, in our study the stabilization of kidney function occurred in a cohort with similar mean eGFR at presentation (49.8 ± 13.4 vs 48.4 ± 34.6 mL/min) but a worse degree of TG (cg score 2.47 ± 0.74 vs 1.57 ± 1.03 and 60% of index biopsies having a cg score of 3) and a higher rate of transplant from deceased donors, especially from extended criteria ones (20% vs 2.8%).

Redfield et al[25] observed that graft survival in 123 patients with cAMR and high chronicity scores was strongly associated with early allograft loss (30% graft survival at 1 year, 20% at 2 years) with a significant impact of higher cg score in graft failure. In the present study, with a median and maximum follow-up time of 20.7 and 38.1 months, respectively, only one patient lost

This article is protected by copyright. All rights reserved

Accepted Article

their graft, even with a high median cg score. Despite the obvious limitations of an observational study and a limited follow-up, the stability of renal function in our TCZ treated population is particularly striking, emphasizing the possible positive role of IL-6 blockade even in the presence of severe glomerular chronic lesions with reduced renal function.

In their recent sub-analysis, Shin et al showed that TCZ reduced total IgG and IgG1-3, and anti-HLA-total IgG and IgG3 levels, suggesting that it non-specifically suppresses Ig production in B cells, likely through inhibition of IL-6-mediated signaling to B cells and plasma cells[31]. In our study TCZ was similarly associated with a significant reduction in anti-HLA DSAs. However, it is well known that a relevant portion of patients with AMR may not exhibit anti-HLA antibodies, nevertheless developing rejection lesions via antibodies directed to other kidney antigens[38,39]. Lefaucheur et al[40] recently showed that AMR patients with AT_1R but not anti-HLA antibodies have a higher prevalence of hypertension, more vascular rejection with arterial inflammation, higher levels of ENDATs, and lack of complement deposition in allograft capillaries, suggesting that AT_1R-Abs may identify kidney transplant recipients at high risk of allograft rejection and loss, independent of the HLA system. Despite these results, clinical effects (also in Lefaucheur et al[40]) have been shown only in a reduced percentage of positive AT_1R patients, and cut-off values were still a matter of debate[40–44]. In our present work we showed that TCZ is also able to reduce AT_1R-Ab levels, a finding that has not been previously reported to the best of our knowledge.

Analyzing histology, TCZ is associated with an early reduction in microvascular inflammation at the 6-month protocol biopsy, reflecting the results of Choi et al[11] at a 1-year biopsy. Additionally, our results also showed the efficacy of IL-6 signaling interference in local inflammation that may potentially anticipate DSA reduction, in particular in the glomerular compartment, where local IL-6 may be produced not only by activated endothelial cells but also by mesangial cells which also express the IL-6 receptor[45–47]. We attempted to analyze these changes at the biopsy gene-expression level, and we observed upregulation of three podocyte and mesangial-related genes (TJP-1, AKR1C3 and CASK) after 6 months of TCZ treatment, which was also confirmed by immunofluorescence. Although confirmation of these results is required in further studies with larger sample sizes, these modifications may reflect a reduction of glomerular inflammation, and the amelioration of the podocyte integrity may further suggest a mitigation of tubular damage, as proposed by other authors[48–51]. Considering the endothelial microenvironment, the absence of significant downregulation in total ENDATs post-TCZ treatment may reflect the need for a longer observation time.

This article is protected by copyright. All rights reserved

In conclusion, treatment with TCZ as a first-line approach for cAMR shows early improvements in serological and histological findings with stable renal function, even in advanced TG. This may support the individual role of TCZ in cAMR without the need for rituximab-based first-line treatments with controversial evidence of effectiveness and potentially negative side effects and should encourage randomized controlled trials on IL-6/IL-6R blockade as a first-line treatment for advanced TG.

Accepted Article

This article is protected by copyright. All rights reserved

FIGURE LEGENDS

Figure 1: Renal function pre- and post–tocilizumab (TCZ) treatment. (A) Median estimated glomerular filtration rate (eGFR) values of TCZ-treated cAMR patients. eGFR was evaluated 3, 6 and 12 months both before and after TCZ treatment (CKD-EPI equation), showing stabilization at the last follow-up (12 months) (eGFR -12 months vs t0, p = 0.002; eGFR t0 vs 12 months, p = 0.006). (B) Median 24-hour proteinuria was unchanged at 6 and 12 months after TCZ treatment (p = 0.410 and 0.925, respectively).

Figure 2: Histological evaluation pre- and post–tocilizumab (TCZ) treatment. (A) Banff scores at diagnosis in patients with available pre- and post-TCZ allograft biopsies. Patients had significant microvascular inflammation (g and ptc) and severe transplant glomerulopathy (cg). (B, a-d) Changes in allograft biopsies before and after TCZ. Significant reduction in microvascular inflammation (g+ptc) (p = 0.019) was observed after TCZ treatment in 6-month protocol biopsies with no progression in chronicity scores (cg and IF/TA).

Figure 3: Immunodominant anti-HLA donor-specific antibody (iDSA) and anti-AT_1R IgG values pre- and post-tocilizumab (TCZ) treatment. (A-B) iDSA mean fluorescence intensity (MFI) values in patients with available protocol biopsies. All iDSAs were of class 2. A significant reduction was observed after 6 months of therapy with TCZ (p = 0.002). (C) A significant reduction in the median concentration of anti-AT_1R IgG was seen after TCZ treatment (p = 0.003).

Figure 4: Gene-expression analysis pre- and post–tocilizumab (TCZ) treatment. (A-B) Relative quantification ($2^{-\Delta\Delta Ct}$) of the expression of ENDAT genes and three mesangial and podocyte marker genes in healthy controls (CTR) compared to patients before and after treatment with TCZ. The upper box and whiskers plots show the distribution of all values from minimum to maximum and the mean for the group of analyzed ENDAT genes. The bar graphs represent geometric mean ± 95% CI. (A) ENDATs were similarly upregulated pre and post TCZ. (B) TJP-1 and AKR1C3 showed a non-significant downregulation in cAMR patients compared to controls and were significantly upregulated after TCZ. CASK was significantly downregulated before TCZ compared to the control group and upregulated after treatment. CASK: Calcium/calmodulin

This article is protected by copyright. All rights reserved

dependent serine protein kinase; TJP-1: Tight junction protein 1; AKR1C3: Aldo-keto reductase family 1 member C3. *: $p < 0.05$; **: $p < 0.01$.

Figure 5: Immunofluorescence assay on kidney biopsies pre- and post–tocilizumab (TCZ) treatment. Pre- and post-treatment sections are stained for TJP-1 (green, A-B), AKR1C3 (C-D) or CASK (red, E-F). In the gene-expression analysis mean fluorescence intensity increased after treatment for TJP-1 (B), AKR1C3 (D), and in this patient also for CASK (F).

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article

REFERENCES

1. Loupy A, Hill GS, Jordan SC. The impact of donor-specific anti-HLA antibodies on late kidney allograft failure. *Nat Rev Nephrol*. 2012;8(6):348-357. doi:10.1038/nrneph.2012.81
2. Billing H, Rieger S, Ovens J, et al. Successful Treatment of Chronic Antibody-Mediated Rejection With IVIG and Rituximab in Pediatric Renal Transplant Recipients. *Transplantation*. 2008;86(9):1214-1221. doi:10.1097/TP.0b013e3181880b35
3. Fehr T, Rüsi B, Fischer A, Hopfer H, Wüthrich RP, Gaspert A. Rituximab and Intravenous Immunoglobulin Treatment of Chronic Antibody-Mediated Kidney Allograft Rejection. *Transplantation*. 2009;87(12):1837-1841. doi:10.1097/TP.0b013e3181a6bac5
4. Hong YA, Kim HG, Choi SR, et al. Effectiveness of rituximab and intravenous immunoglobulin therapy in renal transplant recipients with chronic active antibody-mediated rejection. *Transplant Proc*. 2012;44(1):182-184. doi:10.1016/j.transproceed.2011.12.006
5. Smith RN, Malik F, Goes N, et al. Partial therapeutic response to Rituximab for the treatment of chronic alloantibody mediated rejection of kidney allografts. *Transpl Immunol*. 2012;27(2-3):107-113. doi:10.1016/j.trim.2012.08.005
6. Muller YD, Ghaleb N, Rotman S, et al. Rituximab as monotherapy for the treatment of chronic active antibody-mediated rejection after kidney transplantation. *Transpl Int*. 2018;31(4):451-455. doi:10.1111/tri.13111
7. Mella A, Gallo E, Messina M, et al. Treatment with plasmapheresis, immunoglobulins and rituximab for chronic-active antibody-mediated rejection in kidney transplantation: Clinical, immunological and pathological results. *World J Transplant*. 2018;8(5):178-187. doi:10.5500/wjt.v8.i5.178
8. Moreso F, Crespo M, Ruiz JC, et al. Treatment of chronic antibody mediated rejection with intravenous immunoglobulins and rituximab: A multicenter, prospective, randomized, double-blind clinical trial. *Am J Transplant*. 2018;18(4):927-935. doi:10.1111/ajt.14520
9. Eskandary F, Regele H, Baumann L, et al. A Randomized Trial of Bortezomib in Late Antibody-Mediated Kidney Transplant Rejection. *J Am Soc Nephrol*. 2018;29:591-605. doi:10.1681/ASN.2017070818
10. Kulkarni S, Kirkiles-Smith NC, Deng YH, et al. Eculizumab Therapy for Chronic Antibody-Mediated Injury in Kidney Transplant Recipients: A Pilot Randomized Controlled Trial. *Am J Transplant*. 2017;17(3):682-691. doi:10.1111/ajt.14001

This article is protected by copyright. All rights reserved

Accepted Article

11. Choi J, Aubert O, Vo A, et al. Assessment of Tocilizumab (Anti-Interleukin-6 Receptor Monoclonal) as a Potential Treatment for Chronic Antibody-Mediated Rejection and Transplant Glomerulopathy in HLA-Sensitized Renal Allograft Recipients. *Am J Transplant*. 2017;17(9):2381-2389. doi:10.1111/ajt.14228
12. Sprangers B, Nair V, Launay-Vacher V, Riella L V., Jhaveri KD. Risk factors associated with post-kidney transplant malignancies: An article from the Cancer-Kidney International Network. *Clin Kidney J*. 2018;11(3):315-329. doi:10.1093/ckj/sfx122
13. Chan S, Pascoe EM, Clayton PA, et al. Infection-related mortality in recipients of a kidney transplant in Australia and New Zealand. *Clin J Am Soc Nephrol*. 2019;14(10):1484-1492. doi:10.2215/CJN.03200319
14. Singh P, Ng YH, Unruh M. Kidney Transplantation Among the Elderly: Challenges and Opportunities to Improve Outcomes. *Adv Chronic Kidney Dis*. 2016;23(1):44-50. doi:10.1053/j.ackd.2015.11.002
15. Kodali L, Turner A. When are you too old to get a kidney transplants? *Curr Opin Nephrol Hypertens*. 2019;28(6):593-599. doi:10.1097/MNH.0000000000000548
16. Haugen CE, Thomas AG, Chu NM. Prevalence of Frailty among Kidney Transplant Candidates and Recipients in the United States: Estimates from a National Registry and Multi-center Cohort Study. *Am J Transplant*. 2019 [Epub ahead of print] doi: 10.1111/ajt.15709
17. Haas M, Loupy A, Lefaucheur C, et al. The Banff 2017 Kidney Meeting Report: Revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *Am J Transplant*. 2018;18(2):293-307. doi:10.1111/ajt.14625
18. Sis B, Jhangri GS, Bunnag S, Allanach K, Kaplan B, Halloran PF. Endothelial gene expression in kidney transplants with alloantibody indicates Antibody-mediated damage despite lack of C4d staining. *Am J Transplant*. 2009;9(10):2312-2323. doi:10.1111/j.1600-6143.2009.02761.x
19. Valente V, Teixeira SA, Neder L, et al. Selection of suitable housekeeping genes for expression analysis in glioblastoma using quantitative RT-PCR. *Ann Neurosci*. 2014;21(2):62-63. doi:10.5214/ans.0972.7531.210207
20. Wei R, Stewart EA, Amoaku WM. Suitability of endogenous reference genes for gene expression studies with human intraocular endothelial cells. *BMC Res Notes*. 2013;6:46.

This article is protected by copyright. All rights reserved

doi:10.1186/1756-0500-6-46

21. Madill-Thomsen KS, Wiggins RC, Eskandary F, Böhmig GA, Halloran PF. The Effect of Cortex/Medulla Proportions on Molecular Diagnoses in Kidney Transplant Biopsies: Rejection and Injury Can Be Assessed in Medulla. *Am J Transplant*. 2017;17(8):2117-2128. doi:10.1111/ajt.14233
22. Liu P, Lassén E, Nair V, et al. Transcriptomic and Proteomic Profiling Provides Insight into Mesangial Cell Function in IgA Nephropathy. *J Am Soc Nephrol*. 2017;28(10):2961-2972. doi:10.1681/ASN.2016101103
23. Schindelin J, Arganda-Carrera I, Frise E, et al. Fiji - an Open platform for biological image analysis. *Nat Methods*. 2009;9(7):241. doi:10.1038/nmeth.2019.Fiji
24. Sellarés J, de Freitas DG, Mengel M, et al. Understanding the Causes of Kidney Transplant Failure: The Dominant Role of Antibody-Mediated Rejection and Nonadherence. *Am J Transplant*. 2012;12(2):388-399. doi:10.1111/j.1600-6143.2011.03840.x
25. Redfield RR, Ellis TM, Zhong W, et al. Current outcomes of chronic active antibody mediated rejection - A large single center retrospective review using the updated BANFF 2013 criteria. *Hum Immunol*. 2016;77(4):346-352. doi:10.1016/j.humimm.2016.01.018
26. Rostaing L, Guilbeau-Frugier C, Fort M, Mekhlati L, Kamar N. Treatment of symptomatic transplant glomerulopathy with rituximab. *Transpl Int*. 2009;22(9):906-913. doi:10.1111/j.1432-2277.2009.00896.x
27. Kahwaji J, Sinha A, Toyoda M, et al. Infectious complications in kidney-transplant recipients desensitized with rituximab and intravenous immunoglobulin. *Clin J Am Soc Nephrol*. 2011;6(12):2894-2900. doi:10.2215/CJN.03710411
28. Bachelet T, Nodimar C, Taupin JL, et al. Intravenous immunoglobulins and rituximab therapy for severe transplant glomerulopathy in chronic antibody-mediated rejection: A pilot study. *Clin Transplant*. 2015;29(5):439-446. doi:10.1111/ctr.12535
29. Kahwaji J, Najjar R, Kancherla D, et al. Histopathologic features of transplant glomerulopathy associated with response to therapy with intravenous immune globulin and rituximab. *Clin Transplant*. 2014;28(5):546-553. doi:10.1111/ctr.12345
30. Piñeiro GJ, De Sousa-Amorim E, Solé M, et al. Rituximab, plasma exchange and immunoglobulins: An ineffective treatment for chronic active antibody-mediated rejection. *BMC Nephrol*. 2018;19(1):1-9. doi:10.1186/s12882-018-1057-4
31. Shin B-H, Everly MJ, Zhang H, et al. Impact of Tocilizumab (Anti-IL-6R) Treatment on

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article

Immunoglobulins and Anti-HLA Antibodies in Kidney Transplant Patients with Chronic Antibody-Mediated Rejection. *Transplantation*. 2019:1. doi:10.1097/tp.0000000000002895

32. Choi J et al. Long Term Outcomes Using Tocilizumab For The Treatment Of Chronic Antibody Mediated Rejection . *Am J Transplant*. 2019; 19 (suppl 3)
33. Shin B et al. Clazakizumab (CLZ, Anti-IL-6 Antibody) and Tocilizumab (TCZ, Anti-IL-6 Receptor [r] Antibody) Treatments Differentially Affect IL-6/IL-6R Signaling by Modulating Soluble IL-6R (sIL-6R) and gp130 (sgp130) in Kidney Transplant Patients (KTx Pts) Treated for Chronic Antibody- Mediated Rejection (cABMR) . *Am J Transplant*. 2019; 19 (suppl 3)
34. Patel S, Mohan S, Fernandez H, Batal I, Ratner L, Crew J. Tocilizumab Stabilizes Renal Function in Kidney Transplant Recipients with Chronic Active Antibody Mediated Rejection (CAAMR) . *Am J Transplant*. 2017; 17 (suppl 3)
35. Chandran S, Leung J, Laszik Z, Tang Q, Hu C, Joanes M, Vincenti F. IL-6R Blockade with Tocilizumab Increases Tregs and Decreases Early Graft Inflammation in Kidney Transplant Recipients: Results of a Randomized Controlled Trial . *Am J Transplant*. 2019; 19 (suppl 3)
36. Genberg H, Hansson A, Wernerson A, Wennberg L, Tydén G. Pharmacodynamics of rituximab in kidney allotransplantation. *Am J Transplant*. 2006;6(10):2418-2428. doi:10.1111/j.1600-6143.2006.01497.x
37. Jones JD, Hamilton BJ, Skopelja S, Rigby WFC. Induction of interleukin-6 production by rituximab in human B cells. *Arthritis Rheumatol (Hoboken, NJ)*. 2014;66(11):2938-2946. doi:10.1002/art.38798
38. Zhang Q, Reed EF. The importance of non-HLA antibodies in transplantation. *Nat Rev Nephrol*. 2016;12(8):484-495. doi:10.1038/nrneph.2016.88
39. Philogene MC, Jackson AM. Non-HLA antibodies in transplantation: When do they matter? *Curr Opin Organ Transplant*. 2016;21(4):427-432. doi:10.1097/MOT.0000000000000335
40. Lefaucheur C, Viglietti D, Bouatou Y, et al. Non-HLA agonistic anti-angiotensin II type 1 receptor antibodies induce a distinctive phenotype of antibody-mediated rejection in kidney transplant recipients. *Kidney Int*. 2019:189-201. doi:10.1016/j.kint.2019.01.030
41. Fuss A, Hope CM, Deayton S, et al. C4d-negative antibody-mediated rejection with high anti-angiotensin II type i receptor antibodies in absence of donor-specific antibodies. *Nephrology*. 2015;20(7):467-473. doi:10.1111/nep.12441
42. Malheiro J, Tafulo S, Dias L, et al. Deleterious effect of anti-angiotensin II type 1 receptor

This article is protected by copyright. All rights reserved